Concentration-effect relations of anti-asthma medications. Studies on inflammation markers
Derks, M.G.M.

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Chapter 4

Prevention by theophylline of beta-2-receptor down regulation in healthy subjects

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Chapter 4

ABSTRACT

Objective: Adrenergic down-regulation can occur rapidly in many tissues. Therefore $\beta_2$-agonists might have a rapidly decreasing effect in time, which is a potential problem for the treatment of bronchial asthma. This in vivo study tested the hypothesis that theophylline can prevent adrenergic down-regulation.

Design: A randomised, double blind, placebo-controlled cross-over study was performed in eight healthy subjects. Terbutaline concentration-effect relationships were studied before and after one week of dosing of terbutaline, with or without theophylline.

Methods: Slow-release terbutaline 5 mg daily was administered for 7 days in combination with either placebo or slow-release theophylline. Concentration-effect relationships of terbutaline after a single subcutaneous injection were studied before and after the 7 day terbutaline treatment. Eosinopenia and hypokalemia were the systemic effect parameters. Terbutaline concentration time courses were described with a two-compartment model and those of theophylline with a polynomial equation. A hypothetical effect compartment model was applied to link terbutaline plasma concentration via an $E_{\text{max}}$ model to the studied effects. The interaction of theophylline and terbutaline was described with a non-competitive pharmacodynamic model.

Results: After one week of oral terbutaline, the mean EC$_{50}$ (ng/L) of terbutaline increased for the eosinopenia from $1.87 \pm 1.66$ to $3.78 \pm 2.18$ ($+102\%$) ($p = 0.012$) with placebo, and to $2.73 \pm 1.99$ ($+46\%$) ($p = 0.025$) with theophylline; for the hypokalemia the EC$_{50}$ increased from $4.70 \pm 2.91$ to $8.52 \pm 7.26$ ($+81\%$) ($p = 0.012$) with placebo, and to $5.64 \pm 2.59$ ($+20\%$) ($p = 0.16$) with theophylline.

Conclusion: The results indicate that the non-specific phosphodiesterase inhibitor theophylline can prevent terbutaline-induced adrenergic down-regulation to a substantial degree.

INTRODUCTION

Beta-2-adrenoceptor agonists are used as very effective bronchodilators for the treatment of asthma. Inflammation cells that are active in asthma, like eosinophils
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and neutrophils, express beta-2-adrenoceptors. Therefore, in principle, beta-2-adrenoceptor agonists could influence the function of these cells. The elevation of cAMP in these cells, resulting from beta-2-receptor stimulation, reduces their inflammatory activities. However, studies suggest that the anti-inflammatory effect is present only for a short time due to rapid down-regulation, and that beta-2-adrenoceptor agonists could even exacerbate inflammatory processes due to this down-regulation.

Beta-2-agonists act by binding to the beta2-receptor, stimulating adenylate-cyclase, which via activation of the Gs-protein produce cAMP. cAMP on its turn activates Protein Kinase A (PKA). Consequently, a cascade of phosphorylation of several cellular proteins results in a final action, for example in the lung in relaxation of smooth muscle. However, relaxation can also be obtained via action on presynaptic receptors of cholinergic nerves, which results in inhibition of acetylcholine-release and via the Gs-protein that directly acts on the K+ -channels in the cell membrane, without interference of cAMP. This latter mechanism probably plays an important role in the smooth muscles of the lung. The adrenergic stimulation of leukocytes is mediated via activation of adenylate-cyclase.

Three important mechanisms of adrenergic tachyphylaxis can be discerned. Phosphorylation of the receptor, via kinases that are coupled to the G-protein (GRK), results in uncoupling of the Gs-protein. The same phenomenon can also occur via PKA that is activated by raised concentrations of cAMP. A second mechanism is a reduction of the number of receptors through decreased transcription and/or increased degradation of mRNA. A third possibility is increased degradation of cAMP by induced phosphodiesterase, primarily phosphodiesterase iso-type IV. Induction of phosphodiesterases after beta-2-agonist stimulation has been demonstrated to occur in leukocytes.

Receptor down-regulation due to beta-2-adrenoceptor agonists is of concern for the treatment of asthma. Possible adverse consequences of desensitisation of beta 2-adrenoceptors, such as deterioration of asthma or a bronchoconstriction that is unresponsive to beta-2-adrenoceptor agonists, have been studied with inconclusive results. Investigations on tachyphylaxis have mostly been concerned with pulmonary effects. But a rapid turnover of the receptors and/or
redundancy of receptors ('spare receptors') might prevent down-regulation of clinical importance in the lung to occur. On the other hand, the occurrence and importance of systemic down-regulation has been appreciated, and the reduction in pulmonary beta-adrenoceptor numbers is correlated to those in circulating mononuclear leukocytes. A decrease of the number of spare-receptors can be measured by an increase of the EC\textsubscript{50} of an agonist acting on the receptor. Several mechanisms of action of the still hypothetical prevention by theophylline of beta-2-adrenoceptor down-regulation by theophylline have been suggested by Giembycz. Theoretically, a phosphodiesterase inhibitor can prevent down-regulation of the adrenoceptor that is caused through uncoupling of the receptor or through up-regulation of phosphodiesterase. A recent study did not show theophylline to influence the development of tolerance to the bronchoprotective effect of salmeterol in asthmatics. We are not aware of other \textit{in vivo} studies that show the effect of phosphodiesterase inhibitors on adrenoceptor down-regulation. However, dynamic interactions have been demonstrated \textit{in vitro} between beta-2-agonists and phosphodiesterase inhibitors. Anti-inflammatory actions of phosphodiesterase inhibitors has also been demonstrated.

This study was performed to determine whether theophylline can diminish beta-2-adrenoceptor down-regulation after prolonged administration of terbutaline. We used pharmacokinetic-pharmacodynamic modelling to test the hypothesis that theophylline decreases beta-2-adrenoceptor down-regulation. The dynamic model used has shown to account for the non-competitive agonism of theophylline and terbutaline. Potassium and eosinophil blood concentration were used as systematic effect parameters. Both parameters were shown to be sensitive markers of adrenoceptor activation that can also shown down-regulation.

**MATERIALS AND METHODS**

**Subjects**

The study protocol was approved by the Institutional Review Board of the Academic Medical Center in Amsterdam. Eight healthy Caucasian male students participated in the study. They were non-smoking, and social consumers of alcohol. Routine physical examination, ECG and blood tests were normal.
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age, weight and height were respectively (mean ± SD) 20 ± 1 years, 71 ± 8 kg and 187 ± 9 cm. Written informed consent was obtained.

Design
The study had a double blind, randomised, placebo-controlled, cross-over design. (fig. 1) Terbutaline and theophylline concentration time curves, and their effect on potassium and eosinophil counts were measured at the start and at the end of each period. Two days prior to the start of the first test day, the subjects started taking either placebo or slow release tablets of theophylline, 375 mg for the first dosage and thereafter 250 mg twice daily. This was continued for eleven days, until the morning of the second test day. The theophylline dosage was adjusted after the first test day, when the plasma concentration was outside the aimed range of 5-13 mg/L. On the four test days, the third and eleventh day of both the placebo and the theophylline period, terbutaline was administered subcutaneously, concurrently with the placebo or theophylline intake. On the seven days between two test days, 5 mg slow release terbutaline were given once daily in addition to theophylline or placebo. After the second test day, a wash-out period of minimally eight days was observed before the same schema was repeated, with theophylline and placebo interchanged.

Procedure
The subjects arrived at the hospital at 8.30 am. An indwelling cannula was inserted in a forearm vein and remained in situ during the entire day, enabling frequent blood collection. The system was kept patent with heparinized saline solution with a concentration of 10 U/ml. A light breakfast was allowed after the study medication had been administered. Caffeinated drinks were not allowed the night before and during the test days. A standardised lunch of buns and milk was provided. On each experimental day, before terbutaline administration, blood samples were taken for baseline potassium and eosinophil measurements and to measure the potentially present theophylline concentrations. At t = 0, the subjects were given a subcutaneous dose of 0.0075 mg/kg of terbutaline. At 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 7.5 hours after drug administration, blood samples were taken for measurements of terbutaline and also theophylline plasma concentrations, as well
as plasma potassium levels and peripheral eosinophil counts. Blood samples were centrifuged for 10 minutes at 4000 rpm immediately after collection. Plasma samples for the analysis of terbutaline and theophylline concentrations were stored at -20 °C. Theophylline and terbutaline concentrations were measured at the Dept. of Clinical Pharmacology. The plasma samples for potassium measurements were analysed immediately in the laboratory of the Dept. of Clinical Pharmacology and those for the eosinophil counts were transported as soon as possible to the Laboratory of Clinical Chemistry of the hospital.

<table>
<thead>
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</table>

**Fig. 1. Study design.**

**Dose adjustment**

On the first and third test day, a blood sample for the detection of theophylline that was taken four hours after dosing, was analysed by the pharmacy. Depending on the theophylline blood level the dosage was adjusted for the remainder of the period. The dosage was kept unchanged if the theophylline concentration was within 5-13 mg/l. The pharmacy provided us with sham theophylline concentration, if placebo was given, and this would also result in dose adjustments. If a subject experienced possibly serious drug-related complaints like nausea, he could communicate that to a physician who guarded the blinding of the drugs and who was not directly involved in the experiment, and who could freely decide to reduce the dose with 125 mg per dosage or otherwise, if deemed necessary.
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Test drugs
Theophylline slow-release tablets were Theolair Retard 250 mg (3M Riker); placebo tablets had the same appearance and taste as the theophylline tablets. Terbutaline sulphate for subcutaneous injection was Bricanyl 0.5 mg/L; terbutaline sulphate slow-release tablets were Bricanyl Retard 5 mg (Astra Pharmaceutica).

Terbutaline assay
Plasma levels of terbutaline were analysed using an HPLC method with electrochemical detection as described previously 34.

Theophylline assay
Plasma levels of theophylline were analysed using UV detection as described by Cooper et al 35.

Measurements
For potassium measurements a conventional flame photometer (model 143, Instrumentation Laboratory Inc.) was used. Total blood eosinophil counts were determined with a Technicon H6000 automated differential leukocyte counter (Technicon Instruments Co., Tarrytown, NY) using peroxidase enzyme detection.

Data analysis
Pharmacokinetics of terbutaline
The pharmacokinetic data of terbutaline were fitted to the appropriate equations using the nonlinear regression computer program Scientist 36. A tri-exponential equation describing a two-compartment open model with first order absorption was employed to describe the terbutaline pharmacokinetics 37:

\[ C_t = A e^{-\alpha t} + B e^{-\beta t} - (A + B) e^{-k_e t} \]

Estimates were thus obtained for \( C_t \), the concentration calculated for terbutaline...
(ng/ml) at time t, $k_a$, the absorption rate constant, $\alpha$ and $\beta$, the rate constants of the first, rapid and the second, slower distribution, and $A$ and $B$, their respective intercepts.

From each subject four terbutaline concentration time curves were obtained. Identical $\alpha$ and $\beta$ parameter values were used for these four curves, with only $A$ and $B$ and $K_a$ estimated separately for each curve. The four terbutaline concentration time curves were fitted simultaneously.

**Pharmacokinetics of theophylline**

The theophylline concentrations ($n = 12$) were fitted and described with a polynomial equation: $C_t = a + a_1 \cdot t + a_2 \cdot t^2 + a_3 \cdot t^3$ and so on. The order necessary to describe the data was established by visual inspection and was six at the most.

**Pharmacodynamics**

To relate the terbutaline plasma concentrations (on the placebo days) to the effects on plasma potassium and peripheral eosinophil counts, a sigmoid baseline $E_{max}$ function was applied:

$$E = E_0 \frac{(E_0 - E_{max}) \times C_e^n}{E_C^{50} + C_e^n}$$

In this equation, $E_0$ is the baseline potassium or eosinophil level; $E_C^{50}$ is the $C_e$ that corresponds with 50% of the maximum achievable effect ($E_0 - E_{max}$) and $n$ is the factor expressing the steepness of the concentration-effect relationship. To account for the delay between terbutaline peak plasma concentration ($t_{max}$) and the hypokalemic or eosinopenic nadir, an effect compartment as described by Holford and Sheiner was included in the model. The time course of drug concentrations in the hypothetical effect compartment ($C_e$) for two-compartment kinetics is described by the following equation.
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\[ C_e = \frac{\text{dose} \cdot k_{a2} \cdot k_{e0}}{V_c} \]

\[ \left( \frac{(k_{21} - \alpha)e^{-\alpha t}}{\beta(\alpha - k_0)^{(k_e0 - \alpha)}} \right) + \left( \frac{(k_{21} - \beta)e^{-\beta t}}{(\alpha - \beta)(\alpha - k_a)(k_{e0} - \beta)} \right) + \left( \frac{(k_{21} - k_a)e^{-k_{a0}t}}{(\alpha - k_{e0})(\beta - k_{e0})(k_a - k_{e0})} \right) \]

In this equation, \( V_c \) is the volume of the central compartment, \( k_{e0} \) is the rate constant for the elimination of terbutaline from the effect compartment, \( k_{21} \) is the rate constant for transfer of the drug from the peripheral to the central compartment. The only new parameter in this formula is the \( k_{e0} \), because the \( k_{21} \) and the \( V_c \) can be calculated from the previously estimated PK parameters.

The effect data were fitted to an integrated model, consisting of the \( E_{\text{max}} \) equation and the \( C_e \) equation, using the previously estimated pharmacokinetic parameters of terbutaline as constants. The concentration of theophylline in the effect compartment was described by the differential equation \[ \frac{d(C_e)}{dt} = K_{e0} \ast (C_t - C_e) \]
and fitted by Scientist\(^{36}\).

**Eosinophils**

For the eosinopenic effect induced by terbutaline, \( E_{\text{max}} \)-values were fixed to zero, as in most subjects a tendency of the values towards zero was observed when \( E_{\text{max}} \) was introduced as a free parameter. This had also been observed before\(^{31}\).

To describe the observed effects after the combined administrations of terbutaline together with theophylline, the following equation for non-competitive interaction was used as adapted from Ariens and Simonis\(^{39}\) by van den Berg et al\(^{40}\).
\[ E_{\text{comb}} = E_0^{\text{comb}} \]

\[
\frac{(E_0^{\text{comb}} - E_{\text{max}}^A) \cdot Ce_A^n}{EC50_A^n + Ce_A^n} + \frac{(E_0^{\text{comb}} - E_{\text{max}}^B) \cdot Ce_B^n}{EC50_B^n + Ce_B^n}
\]

\[
\left( \frac{(E_0^{\text{comb}} - E_{\text{max}}^A) \cdot Ce_A^n}{EC50_A^n + Ce_A^n} + \frac{(E_0^{\text{comb}} - E_{\text{max}}^B) \cdot Ce_B^n}{EC50_B^n + Ce_B^n} \right) - \frac{E_{0^{\text{comb}} - E_m}}{E_{0^{\text{comb}} - E_m}}
\]

In this equation \( E_{\text{comb}} \) is the effect of the combined terbutaline and theophylline administration, \( E_0^{\text{comb}} \) is the baseline of the combined effect. Parameters for one agonist are indicated by A, for the other drug by B. \( E_m \) is the hypothetical maximum achievable effect of the receptor effector system.

**Potassium**

For the description of the effects on plasma potassium we used the same model as for describing the eosinophil concentrations. \( E_m, E_0^{\text{comb}}, E_{\text{max}}^A \) and \( E_{\text{max}}^B \) were estimated by the fitting program. Thus, unlike the modelling of eosinopenic effects after single drug administration of terbutaline, \( E_{\text{max}}^A, E_{\text{max}}^B \) and consequently the maximum achievable effect of the system for the combined drugs, \( E_m \), were free parameters and not fixed at zero.

We expected spare-receptors to be present, and in order to increase power, we kept the \( E_{\text{max}} \) values constant on all four test days and allowed only the \( EC_{50} \) values of terbutaline to change. We assumed that the \( EC_{50} \) of terbutaline on the first day in the placebo period was identical to the \( EC_{50} \) on the first day in the theophylline period. Secondly, we assumed that the \( EC_{50} \) of theophylline did not change during the seven day treatment period. A change in the difference between
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EC₅₀ values of terbutaline on the first and last test day for the two periods can so be attributed to the effect of theophylline.

We allowed the baseline values for each subject to be different on all four days, as the data in each of the two periods for both studied effects supported this. The Kₑ₀, Eₘₐₓ and n values of terbutaline were assumed to be identical on all four test days but they were estimated for each subject by simultaneously fitting only the two effect curves in the placebo period when theophylline drug interaction had not to be accounted for.

The EC₅₀, Kₑ₀, Eₘₐₓ and n values of theophylline and the Eₘ value for each of the two studied effects on the two test days were assumed to be identical and estimated per individual in a simultaneous fitting procedure for each pair of effect curves.

Statistical analysis
Comparisons of pharmacodynamic parameters for the effects on potassium and eosinophils were made with the two-tailed Wilcoxon Signed Ranks test for paired data. The goodness of fit was expressed by the coefficient of determination (CoD) and was calculated by Scientist.

RESULTS
None of the subjects experienced any serious adverse effect during the time the study lasted.

Kinetics
Terbutaline
The estimated kinetic parameters for the terbutaline and the mean coefficient of determination of the four curves are presented in table I. For the four absorption rate half-lives and Vₑ's, the mean and standard deviation are given. On all four days, terbutaline was not present in pre-dose samples.

Theophylline
The mean theophylline concentrations for the two test days during the
theophylline period and the mean coefficient of determinations are presented in table II. Theophylline was lowered twice to 125 mg twice daily according to the protocol (concentration between 13 and 20 mg/L); the placebo was reduced three times with the same dose adjustment.

**Dynamics**
The effect curves were satisfyingly described by the models, judged by visual inspection. The EC\(_{50}\) values of terbutaline for the first test day in the placebo period (EC\(_{50}\) pl1), and for the last test days of respectively the placebo period (EC\(_{50}\) pl2) and the theophylline period (EC\(_{50}\) comb) are presented in table IIIa for the hypokalemic effect and in IVa for the eosinopenic effect. The other dynamic parameters for terbutaline, including the mean estimated values for baseline on the two placebo test days and the mean coefficient of determination as calculated from the two curves obtained in 8 subjects during the placebo period are presented in table IIIb for the hypokalemic effect and in IVb for the eosinopenic effect. No systematic difference in goodness of fit between the four days existed. The mean values (± SD) for the hypothetical maximum achievable effect of the receptor effector system (for the hypokalemic effect), the dynamic parameters to describe the contribution of theophylline to the effect and the mean coefficient of determination as calculated from the 2 curves obtained in 8 subjects during the theophylline period are presented in table IIIc for the hypokalemic effect and in IVc for the eosinopenic effect. Fits of the combined drug effect on potassium and eosinophils of a representative subject (subj. 1) are shown in figure 2 and figure 3, respectively.

**Potassium**
The EC\(_{50}\) of terbutaline for the hypokalemic effect was increased from 4.7 ± 2.9 μg/L on the first test day of the placebo period to 8.5 ± 6.3 μg/L on the final day of the placebo period (p = 0.012). The concomitant dosing of theophylline resulted in a value for the EC\(_{50}\) on the final day of 5.6 ± 2.6 mg/L. This EC\(_{50}\) did not significantly differ from the EC\(_{50}\) at the start of the placebo period (p = 0.16). But the difference from the EC\(_{50}\) after the placebo period, although substantial in several subjects, was also not statistically significant (p = 0.31). As was already mentioned above, the EC\(_{50}\) of terbutaline on the first theophylline day was set at
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the value found on the first placebo day.

Table 1. Individual kinetic parameters after subcutaneous injection of 0.0075 mg/kg terbutaline, using a two-compartment model.

<table>
<thead>
<tr>
<th>Subject</th>
<th>aK-T1/2 (SD) (h)</th>
<th>a-T1/2 (h)</th>
<th>β-T1/2 (h)</th>
<th>K21-T1/2 (h)</th>
<th>Vc (SD) (L)</th>
<th>CoD</th>
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<td>0.23 (0.04)</td>
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<td>40 (14)</td>
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<td>2</td>
<td>0.25 (0.12)</td>
<td>0.29</td>
<td>3.34</td>
<td>1.02</td>
<td>40 (17)</td>
<td>0.9</td>
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<td>3</td>
<td>1.07 (0.09)</td>
<td>0.17</td>
<td>3.66</td>
<td>3.52</td>
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<td>0.17 (0.10)</td>
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<td>1.53</td>
<td>0.55</td>
<td>81 (16)</td>
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<td>5</td>
<td>0.26 (0.09)</td>
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<td>3.1</td>
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aK-T1/2 and Vc are mean values of the four test days.

Eosinophils
The EC50 of terbutaline for the eosinopenic effect was increased from 1.9 ± 1.7 µg/L on the first day of the placebo period to 3.8 ± 2.2 µg/L on the final day of the placebo period (p = 0.012). The concomitant dosing of theophylline resulted in a smaller increase of EC50 on the final day of the test period, being 2.7 ± 2.0 mg/L; the EC50 was larger than the EC50 at the start of the placebo period (p = 0.025), but was smaller than the EC50 at the end of the placebo period (p = 0.012). As was
already mentioned above, the EC50 of terbutaline on the first theophylline day was set at the value found on the first placebo day.

**Table 2.** Mean theophylline concentrations after repeat administration of twice daily 250 mg slow-release theophylline. For subjects 1 and 7 the dose was reduced to 125 mg after day 1.

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<th>day 2 (mg/L)</th>
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<td>9.8</td>
<td>7.2</td>
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<td>6</td>
<td>11.3</td>
<td>8.1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>12.7</td>
<td>6.9</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>9.8</td>
<td>9.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Mean</td>
<td>9.6</td>
<td>7.2</td>
<td>1</td>
</tr>
<tr>
<td>SD</td>
<td>2.7</td>
<td>1.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

a CoD is mean value for the two days.
Fig. 2. Representative of fits of the hypokalemic effect of terbutaline and theophylline combined on the first day (solid circles) and the last day (open squares). (subj.1)
Fig. 3. Representative of fits of the eosinopenic effect of terbutaline and theophylline combined on the first day (solid circles) and the last day (open squares). (subj. 1)
Prevention of adrenergic down-regulation by theophylline

Table 3a. EC₅₀ values of subcutaneous terbutaline for the hypokalemic effect; pre-treatment (EC₅₀_pll), after repeat administration of slow-release terbutaline 5 mg (EC₅₀_pll₂), and after repeat administration of slow-release 250 theophylline twice daily and repeat administration of 5 mg slow-release terbutaline (EC₅₀_comb). (The theophylline dose was reduced for subjects 1 and 7.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>EC₅₀_pll (µg/L)</th>
<th>EC₅₀_pll₂ (µg/L)</th>
<th>EC₅₀_comb (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.57</td>
<td>5.02</td>
<td>4.99</td>
</tr>
<tr>
<td>2</td>
<td>5.68</td>
<td>11.30</td>
<td>4.97</td>
</tr>
<tr>
<td>3</td>
<td>2.42</td>
<td>3.57</td>
<td>4.52</td>
</tr>
<tr>
<td>4</td>
<td>2.84</td>
<td>4.09</td>
<td>2.44</td>
</tr>
<tr>
<td>5</td>
<td>2.29</td>
<td>2.76</td>
<td>2.80</td>
</tr>
<tr>
<td>6</td>
<td>10.85</td>
<td>25.00</td>
<td>9.36</td>
</tr>
<tr>
<td>7</td>
<td>5.79</td>
<td>8.12</td>
<td>7.29</td>
</tr>
<tr>
<td>8</td>
<td>5.12</td>
<td>8.31</td>
<td>8.78</td>
</tr>
</tbody>
</table>

Mean 4.70 8.52† 5.64‡
SD 2.91 7.26 2.59

DISCUSSION

Theophylline in therapeutic concentrations given concomitantly with terbutaline partly prevented the increase of the EC₅₀ of terbutaline for the hypokalemic and eosinopenic effect in healthy subjects. Failure to reduce the down-regulation did not occur in case of the eosinopenic effect, and in three of the subjects in case of the hypokalemia. Several matters have to be considered for a correct interpretation of the results that we obtained with the non-competitive agonist
Chapter 4

drug-effect model.

Spare receptors have been shown to be present for beta-2-adrenoceptors \(^{18}\). Down regulation of these spare receptors will result in a lower EC\(_{50}\) for beta-2-adrenoceptor agonists \(^{19}\). With PK/PD modelling as applied in this study it is not possible to discern differential effects on sensitivity (EC\(_{50}\)) and at the same time effect on maximal achievable effect (E\(_{\text{max}}\)), i.e. a pure receptor down-regulation in a situation where no 'spare'-receptors are present. Pragmatically we chose to study a possible difference in the EC\(_{50}\) and not also in E\(_{\text{max}}\). For eosinophils the E\(_{\text{max}}\) had previously shown to be 0, and therefore this parameter was fixed at and any down regulation could consequently only be expressed in our model in change of EC\(_{50}\). However, these phenomena can occur independently of each other \(^{41},42\).

The model only takes into account that EC\(_{50}\) and not that also the E\(_{\text{max}}\) could possibly change. This approach was chosen for the analysis of data, because otherwise we would have had too many parameters for the curve fitting procedure, which would have obscured the detection of EC\(_{50}\) changes. The dynamic model we used for the combined effect of two drugs, assumes a shared pathway of the intracellular response system of theophylline and terbutaline. This model has been shown to give better effect descriptions than a mere summation of the effects of a beta-agonist (formoterol) and theophylline \(^{31}\). It would have been more precise to have obtained the value from single drug effect-curves. We were confident though that the model would be able to provide us with reliable estimations, as it was shown in a previous study to be capable of doing so \(^{31}\). It was also assumed that the EC\(_{50}\) of theophylline did not change over the two days. This is a realistic assumption as theophylline had already been given for two days at the start of the first test days.

The estimation of the K\(_{e0}\) and the sigmoid for the theophylline was in some subjects difficult because the concentration was at steady state. Furthermore, the concentration was never above the EC\(_{50}\), while the concentration effect relationship appeared to be rather steep with a mean sigmoid factor of approximately 6. By fitting the two theophylline curves in one fitting procedure, with common K\(_{e0}\), EC\(_{50}\) and sigmoid factor, this problem was solved. Besides, the results appeared to agree with earlier finding of single dose studies, in which higher concentrations were obtained \(^{31}\).
Phosphodiesterases catalyse the degradation of the cyclic nucleotides cAMP and cGMP. In human leukocytes iso-type 4 is predominantly present.\(^{43}\) Theophylline, a non-specific phosphodiesterase inhibitor is a bronchodilator, but anti-inflammatory effects may also contribute to its action in asthma control.\(^{44-48}\) Recently, clinical trials indicated that the addition to corticosteroids of either the long-acting beta-2-agonist formoterol, or theophylline have a favourable effect on symptoms of asthma.\(^{49-51}\)

In conclusion, beta-2-agonists in combination with theophylline in the low-therapeutic concentration range can possibly provide an anti-inflammatory effect in addition to its bronchodilatory action.

**Table 3b.** Mean dynamic parameters of all subjects for the hypokalemic effect of terbutaline.

<table>
<thead>
<tr>
<th></th>
<th>(^aE_{0, pl})</th>
<th>(E_{\max})</th>
<th>(K_{\infty})</th>
<th>(n)</th>
<th>(^aCoD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mmol/L)</td>
<td>(mmol/L)</td>
<td>(h(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.9</td>
<td>2.6</td>
<td>6.0</td>
<td>2.80</td>
<td>0.6</td>
</tr>
<tr>
<td>SD</td>
<td>0.1</td>
<td>0.4</td>
<td>1.9</td>
<td>0.80</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(^aE_{0\, pl}\) and CoD are mean values of the two placebo days.

**Table 3c.** Mean dynamic parameters of all subjects for the hypokalemic effect of theophylline.

<table>
<thead>
<tr>
<th></th>
<th>(^aE_{0, \text{comb}})</th>
<th>(EC_{50, \text{th}})</th>
<th>(n_{d, d})</th>
<th>(K_{\infty, dh})</th>
<th>(E_{m})</th>
<th>(^aCoD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mmol/L)</td>
<td>(mg/L)</td>
<td></td>
<td>(h(^{-1}))</td>
<td>(mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.0</td>
<td>22.6</td>
<td>3.4</td>
<td>2.5</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>SD</td>
<td>0.2</td>
<td>11.9</td>
<td>2.1</td>
<td>1.9</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(^aE_{0\, \text{comb}}\) and CoD are mean values of the two theophylline days.
Table 4a. EC$_{50}$ values of terbutaline for the eosinopenic effect; pre-treatment (EC$_{50 \text{pl}1}$), after repeat administration of slow-release terbutaline 5 mg (EC$_{50 \text{pl}2}$), and after repeat administration of slow-release 250 theophylline twice daily and repeat administration of 5 mg slow-release terbutaline (EC$_{50 \text{comb}}$). (The theophylline dose was reduced for subjects 1 and 7.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>EC$_{50 \text{pl}1}$ ($\mu$g/L)</th>
<th>EC$_{50 \text{pl}2}$ ($\mu$g/L)</th>
<th>EC$_{50 \text{comb}}$ ($\mu$g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.11</td>
<td>4.57</td>
<td>1.89</td>
</tr>
<tr>
<td>2</td>
<td>2.37</td>
<td>3.53</td>
<td>2.81</td>
</tr>
<tr>
<td>3</td>
<td>1.27</td>
<td>2.66</td>
<td>2.53</td>
</tr>
<tr>
<td>4</td>
<td>0.35</td>
<td>2.00</td>
<td>1.59</td>
</tr>
<tr>
<td>5</td>
<td>0.89</td>
<td>1.41</td>
<td>0.99</td>
</tr>
<tr>
<td>6</td>
<td>1.54</td>
<td>5.05</td>
<td>3.08</td>
</tr>
<tr>
<td>7</td>
<td>1.73</td>
<td>2.77</td>
<td>1.60</td>
</tr>
<tr>
<td>8</td>
<td>5.70</td>
<td>8.24</td>
<td>7.33</td>
</tr>
<tr>
<td>Mean</td>
<td>1.87</td>
<td>3.78†</td>
<td>2.73†</td>
</tr>
<tr>
<td>SD</td>
<td>1.66</td>
<td>2.18</td>
<td>1.99</td>
</tr>
</tbody>
</table>

p < 0.05†

Table 4b. Mean dynamic parameters of all subjects for the eosinopenic effect of terbutaline.

<table>
<thead>
<tr>
<th>aE$_{0\text{pl}}$ (10$^{-6}$/L)</th>
<th>K$_{e0}$ (h$^{-1}$)</th>
<th>n</th>
<th>aCoD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>164</td>
<td>0.6</td>
<td>1.19</td>
</tr>
<tr>
<td>SD</td>
<td>90</td>
<td>0.8</td>
<td>0.45</td>
</tr>
</tbody>
</table>

aE$_{0\text{pl}}$ and CoD are mean values of the two placebo days.
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Table 4c. Mean dynamic parameters of all subjects of theophylline for the eosinopenic effect.

<table>
<thead>
<tr>
<th></th>
<th>$a E_{0\text{comb}}$ ($10^{-6}$/L)</th>
<th>EC$_{50\text{th}}$ (mg/L)</th>
<th>$n_{\text{th}}$</th>
<th>K$_{50\text{th}}$ (h$^{-1}$)</th>
<th>$a$ CoD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>196</td>
<td>13.4</td>
<td>6.2</td>
<td>3.3</td>
<td>0.7</td>
</tr>
<tr>
<td>SD</td>
<td>89</td>
<td>2.2</td>
<td>3.1</td>
<td>3.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

$E_{0\text{comb}}$ and CoD are mean values of the two theophylline days.

ACKNOWLEDGMENTS

We want to thank Henk-Jan Guchelaar of the pharmacy of the AMC for the measurements of the theophylline concentrations used for the adjustment of the dose, and Mrs. Els Portier of the laboratory of the Department of Clinical Pharmacology for skilfully measuring the terbutaline and theophylline concentrations. We also would like to thank 3M Pharmaceuticals for providing us with the placebo tablets.

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